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Animal Models in Q Fever: Pathological Responses of Inbred Mice to Phase I *Coxiella burnetii*

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The susceptibility of inbred strains of mice to infection by phase I *Coxiella burnetii*, the aetiological agent of Q fever, was investigated by evaluating morbidity, mortality, antibody production and *in vitro* proliferative responses of splenic lymphocytes. Among the 47 strains of mice tested for morbidity and mortality to *C. burnetii* infection, 33 were resistant, 10 were of intermediate sensitivity, and four were sensitive. A/J mice exhibited the highest mortality, and surviving mice of this strain yielded high concentrations of viable rickettsiae from essentially all organs for more than 3 weeks after inoculation. However, A/J mice developed a protective immune response after vaccination with inactivated *C. burnetii* cells. Induction of gross pathological responses and antibody production were similar in sensitive mice (strain A/J) and resistant mice (strain C57BL/6J). The LD₅₀ of phase I *C. burnetii* for A/J mice was about 1000-fold lower than that for the more resistant C57BL/6J mice. Mice of both strains developed antibody titres against phase I cells, phase II cells, and phase I lipopolysaccharide after the injection of one or more viable phase I organisms of *C. burnetii*; five or more rickettsiae caused splenomegaly that was almost proportional to the infecting dose. Suppression of *in vitro* proliferative responses of splenic lymphocytes to concanavalin A, a T-cell mitogen, was apparent after infection of sensitive A/J mice with as few as one to five phase I micro-organisms. However, suppression of proliferation of splenic lymphocytes from resistant C57BL/6J mice required 10⁷ phase I *C. burnetii*.

INTRODUCTION

The quest for an immunologically unaltered animal that exhibits the spectrum of pathogenetic reactions caused by *Coxiella burnetii*, the aetiological agent of Q fever, with a concomitant significant mortality rate, was the subject of the current study. Hosts involved in the epizootiology of Q fever range from animal ectoparasites to man (Ormsbee, 1965). A micro-organism adapted to growth in so many species has probably evolved a strategy of peaceful co-existence (Hackstadt & Williams, 1981). Since the discovery of Q fever (Derrick, 1937) extensive studies on the infectivity of *C. burnetii* for various animals have been undertaken. Although one micro-organism is sufficient to cause Q fever in small laboratory animals used for experimental purposes, pathogenic reactions depend on the virulence of the strain and the dose inoculated (Hackstadt & Williams, 1981; Ormsbee *et al.*, 1978). Mice and guinea pigs are used routinely as experimental animals for pathogenesis and immunogenesis studies with phase I *C. burnetii* (Ormsbee, 1965; Paretsky *et al.*, 1964). Although both species are highly susceptible,

Abbreviations: Con A, concanavalin A; IFA, indirect fluorescent antibody; i.p., intraperitoneally; LPS-I, phase I lipopolysaccharide.

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infected animals rarely demonstrate morbidity. Mortality occurs in mice only when large doses of viable or inactivated phase I *C. burnetii* are administered (Williams & Cantrell, 1981).

The availability of inbred mice strains carrying resistance genes to a number of bacterial and viral infections has refined the experimental approaches for the development of small animal models for studying various infectious diseases (Cheers & McKenzie, 1978; Lopez, 1975; Plant & Glynn, 1976). Genes outside the H-2 complex controlling susceptibility to various infectious diseases are thought to represent mechanisms other than those involving specific immune recognition (Groves *et al.*, 1980; Skamene *et al.*, 1979). Patterns of susceptibility among inbred and outbred strains of mice to lethal infections with *Rickettsia tsutsugamushi* are mouse-strain-dependent (Groves & Osterman, 1978). Natural resistance to the Gilliam strain of *R. tsutsugamushi* is controlled by a single autosomal dominant gene unlinked to the H-2 complex (Groves *et al.*, 1980). Susceptibility of inbred mouse strains to *R. akari*, the aetiological agent of rickettsial pox, was not easily correlated with a specific genotype, thus demonstrating a wide range of pathogenic responses in various mice. Among the strains surveyed, resistant strains survived a challenge dose of 10^5 more rickettsiae than the lethal dose for sensitive strains (Anderson & Osterman, 1980). Although *R. akari* is markedly different from *C. burnetii*, these studies suggest that selected inbred strains of mice might exhibit increased sensitivity to infections by phase I *C. burnetii*.

In the present study, inbred strains of mice were tested for morbidity and mortality after infection by phase I *C. burnetii*. The dissemination and replication of *C. burnetii* in the most sensitive strain (A/J), and the ability of this strain to mount a protective immune response after vaccination were examined. The effects of *C. burnetii* infection in a resistant (C57BL/6J) and a sensitive (A/J) strain were compared after administration of varying doses of phase I rickettsiae. Gross pathological responses, the production of antibodies against phase I and phase II whole cells and against the phase I lipopolysaccharide (LPS-I), and the modulation of *in vitro* proliferative responses of splenic lymphocytes from these representative strains were examined.

METHODS

Mice. Forty-seven strains of inbred female mice, 6-7 weeks old, were evaluated. Mice were purchased from the Jackson Laboratory, Bar Harbor, Maine, USA; Charles River Breeding Laboratories Inc., Wilmington, Mass., USA; and the Division of Research Services, National Institutes of Health, Bethesda, Md., USA. Prior to use samples of mice from each shipment were examined for histopathological lesions, and tested serologically for 10 common mouse pathogens including lymphocytic choriomeningitis, sendai and mouse hepatitis virus. Mice were housed 10 per cage in a ventilated room at 23-24 °C. Commercial laboratory diet and water were provided *ad libitum*.

***C. burnetii*.** The Nine Mile isolate of *C. burnetii* in phase I (fourth egg passage) was prepared as a 50% (w/v) yolk-sac suspension in brain heart infusion broth (BHI), shell-frozen, and stored at -70 °C.

Evaluation of sensitive and resistant strains. Groups of 10 mice from each strain were inoculated intraperitoneally (i.p.) with $10^{6.5}$ rickettsiae contained in 0.2 ml BHI. Mice were observed daily for morbidity (visually expressed as roughened fur, lethargy, weakness, huddling) and mortality during a 28 d post-injection period. Mice that survived for 28 d were exsanguinated and their sera were pooled by groups for the determination of anti-*C. burnetii* antibodies. Strains of mice that exhibited neither overt signs of morbidity nor mortality were classified resistant. Strains in which the inoculated mice became moribund, but suffered no mortalities, were classified as being of intermediate sensitivity to infection, and strains in which mortalities occurred were considered sensitive.

Vaccine. Formalin-inactivated, phase I whole-cell vaccine was prepared initially for human use from the Henzerling strain of *C. burnetii* by Merrell-National Laboratories, Swiftwater, Pa., USA, and designated NDBR 105. Procedures for the growth of the *C. burnetii* in yolk-sacs of embryonated chicken eggs, inactivation with formalin, and purification were as previously described (Spicer & DeSanctis, 1976). Purified *C. burnetii* were suspended (1 mg ml^{-1}) in phosphate-buffered saline (PBS; 0.15 M-sodium chloride, 0.15 M-sodium phosphate, pH 7.0) containing 0.01% (w/v) merthiolate, diluted to $120 \mu\text{g}$ micro-organisms (dry wt) ml^{-1} with 4% (w/v) lactose, and freeze-dried. The vaccine was reconstituted in sterile physiological saline (0.85% w/v) for injection.

Antibody measurements. Sera from mice given injections of either viable phase I or killed *C. burnetii* vaccine were tested for antibody against phase II *C. burnetii* by the indirect fluorescent antibody (IFA) method (Philip & Casper, 1978). Antibodies against phase I and phase II whole cells and LPS-I were quantified by ELISA (Williams *et al.*, 1986). Titres were expressed as the reciprocals of the highest serum dilution that gave a positive reaction.

Titration of rickettsiae in infected tissues. Tissues were prepared as 10% (w/v) BHI homogenates. Groups of seven

mice were inoculated i.p. with 0.2 ml from serial 10-fold dilutions of the homogenates. Mice surviving for 28 d were exsanguinated and the individual sera were tested for antibodies to *C. burnetii*. Mice that died or seroconverted were considered infected and the median infective dose (MID_{50}) for each sample was calculated using the moving average method (Thompson, 1947). One *C. burnetii* organism is required to establish infection in mice (Ormsbee *et al.*, 1978).

Lymphocyte proliferation assays At various times after infection, mice were bled from the orbital venous plexus, then killed by cervical dislocation. Spleens were aseptically removed and weighed, and organs were inspected for signs of gross necrotic foci. After the spleen weights had been determined, single-cell suspensions of spleens from control and test mice were prepared by gentle disruption through a wire screen into ice-cold RPMI 1640 medium (Moore *et al.*, 1967) containing 5% (v/v) heat-inactivated foetal calf serum, 50 μ M-2-mercaptoethanol, and 50 μ g gentamicin sulphate ml^{-1} . Spleen cells were washed twice in cold RPMI medium and adjusted to a concentration of 2×10^6 nucleated cells ml^{-1} . Lymphocyte proliferation assays were done using a microculture assay procedure described by Damrow *et al.* (1985). Cultures were stimulated *in vitro* with either concanavalin A (Con A) at 1.0 μ g ml^{-1} , or with killed whole cells of phase I *C. burnetii* as recall antigen at 5 μ g (dry wt) ml^{-1} . The proliferative responses of lymphocytes were expressed as stimulation indices (SI) computed as follows:

$$SI = \frac{\text{c.p.m. (mitogen- or antigen-stimulated)}}{\text{c.p.m. (unstimulated controls)}}$$

RESULTS

Infection of mice with *C. burnetii*

Observation of morbidity and mortality in inbred strains of mice after i.p. injection of *C. burnetii* indicated that mice in 14 of the 47 strains tested became overtly ill (Table 1). The majority (90–100%) of mice in substrains of the A, BALB, and 129 lines became ill within 3–6 d of inoculation and the average duration of illness was 3–17 d. Comparison of the strains based on the numbers ill and duration of illness showed no correlation with major histocompatibility complex (MHC) genes. Mortalities were observed in the H-2^a, H-2^q and H-2^d haplotypes, but other strains with the same haplotypes were resistant. Although the incidence of mortality

Table 1. Responses of inbred strains of mice to i.p. inoculation with $10^{6.5}$ phase I *C. burnetii*, Nine Mile isolate

Ten of each mouse strain were inoculated. Observations were terminated after 28 d.

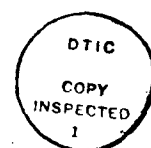
Mouse strain	H-2 haplotype	Overt illness*		No. mice dead/total	IFA titre†
		Percentage of mice affected	Mean duration (d)		
A/J	a	100	4.0	7/10	1280
A/HeJ	a	100	3.5	0/10	640
A/WySnJ	a	90	6.5	3/10	1280
AU/SsJ	q	40	7.8	3/10	640
BALB/cByJ	d	90	10.4	0/10	1280
BALB/cDub	—	100	4.2	0/10	1280
BALB/cJ	d	100	7.0	0/11	1280
BALB/cNcrI Br	—	100	17.0	0/10	2560
BDP/J	p	70	3.6	0/10	ND
C3H/HeJ	k	50	3.1	0/10	640
NZB/BinJ	d	40	2.6	2/10	640
LG/J	d	20	3.0	0/10	2560
129/SvJ	b	90	5.9	0/10	2560
129/J	b	90	8.3	0/10	1280

ND, Not done.

—, Haplotype unknown.

* Mice with overt illness were lethargic and had rough fur.

† Pool of sera from survivors; reciprocal of highest positive dilution.



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Table 2. *Inbred strains of mice that exhibited no overt signs of illness after i.p. injection of phase I C. burnetii*

Mouse strain	H-2 haplo-type	IFA titre*	Mouse strain	H-2 haplo-type	IFA titre*
AKR/J	k	2560	C57L/J	b	640
CBA/CaJ	k	1280	C57/6FMaiBR	-	1280
CBA/J	k	2560	C58/J	k	1280
CBA/HT6J	k	2560	DBA/1J	q	1280
CE/J	k	ND	DBA/2J	d	2560
C3H/HeN	k	1280	DBA/2FMaiBR	-	1280
C3H/HeSnJ	k	1280	1/LnJ	j	1280
C3H/Rv	-	1280	MA/MyJ	k	1280
C3H/HeDub	k	640	PL/J	u	ND
C3H/BiMaiBR	-	640	RF/J	k	2560
CeHeB/FeJ	k	2560	RIIS/J	r	1280
C57BL/KSJ	d	1280	SEA/GnJ	d	ND
C57BL/6J	b	2560	SEC/ReJ	d	1280
C57BL/10J	b	2560	SJL/J	s	2560
C57BL/10SnJ	b	1260	ST/bJ	k	2560
C57BL/6ByJ	b	2560	SWR/J	q	1280
C57BR/cdJ	k	320			

ND, Not done.

-, Haplotype unknown.

* Pool of sera from survivors; reciprocal of the highest positive dilution.

varied in separate experiments, the highest incidence consistently occurred in A/J mice. Quality assurance testing on the mouse populations indicated that this variation could not be attributed to adventitious agents.

Thirty-three strains without overt signs of illness after infection were classified as resistant (Table 2). Although antibody titres in resistant strains were higher than would be expected without replication of the injected organisms, these mice remained healthy and vigorous throughout the 28 d observation period. Antibody titres in surviving mice from the various strains ranged from 320 to 2560. There were no consistent differences among antibody titres in strains with inapparent infections and titres in those that became ill. No correlation was observed between antibody titre and H-2 haplotype.

After infection of the sensitive A/J mice *C. burnetii* were detected in every organ examined. Viable *C. burnetii* in various tissues were titrated at intervals from 4 to 21 d after the mice were inoculated. Blood samples taken 4 d after inoculation contained more than $10^{3.4}$ *C. burnetii* ml⁻¹ and even higher titres ($> 10^5$) were present in other tissues. The following peak concentrations (number of micro-organisms per ml or per g of tissue) occurred 7 d after inoculation: whole blood, $10^{4.2}$; heart, $10^{7.3}$; liver, $10^{7.5}$; lung, $10^{7.3}$; spleen, $10^{6.8}$; kidney, $10^{7.9}$; brain, $10^{5.7}$. Estimates of the tissue burdens were of course somewhat confounded by the presence of *C. burnetii* in whole blood. However, after 7 d the numbers of viable *C. burnetii* in all organs began to decrease. Concentrations ranging from $10^{2.7}$ (g brain)⁻¹ to $10^{5.7}$ (g spleen)⁻¹ were present 3 weeks after the mice were infected.

Protective immune response in vaccinated A/J mice

Since A/J mice were the most sensitive of the strains examined, the ability of this strain to develop a protective immune response against virulent *C. burnetii* challenges was determined. Three groups of mice were given a single 0.2 ml subcutaneous injection that contained either 0.25, 2.5 or 25.0 µg vaccine. Animals in a fourth group received two subcutaneous injections each containing 12.5 µg vaccine at a 7 d interval. Control mice were injected twice with saline. Eight weeks after injection, mice from each group were challenged i.p. with 10^7 phase I *C. burnetii*, Nine Mile strain.

Table 3. Antibody titres in the sera of A/J mice injected with inactivated phase I *C. burnetii* vaccine

Time of titration (weeks post- vaccination)	Vaccine dose (μ g)	IFA titre*			
		25†	25	2.5	0.25
2		106 \pm 27	426 \pm 106	67 \pm 13	13 \pm 3
4		213 \pm 53	160 \pm 0	67 \pm 13	<10 \pm 0
8		640 \pm 0	133 \pm 27	13 \pm 3	<10 \pm 0

* Mean \pm standard error of titres from three mice.

† Given in two equal subcutaneous injections, 7 d apart. All other doses were given as a single injection.

Table 4. Effect of vaccination on the resistance of A/J mice to *C. burnetii* infectionA dose of 10^7 MID₅₀ phase I *C. burnetii* was injected i.p. 8 weeks after vaccination.

Vaccine dose (μ g)	IFA titre*	No. of mice responding/total	
		With overt illness	Dead
None	0	57/57	23/57
0.25	<10 \pm 0	3/26	2/26
2.5	13 \pm 3	0/27	0/27
25	133 \pm 27	0/27	0/27
25†	640 \pm 0	0/30	0/30

* Mean \pm standard error of titres from three mice.

† Given as two equal subcutaneous injections, 7 d apart. All other doses were given as a single injection.

Antibody titres elicited in vaccinated mice were related to the amount of vaccine injected and whether it was administered in a single or divided dose (Table 3). Administering the vaccine in two separate injections given 7 d apart elicited higher and more stable titres than the same amount given as a single dose. Titres in mice that received the divided vaccine dose were still at peak levels after 2 months, while titres in mice that received the same amount of antigen in a single injection started to decline within 4 weeks. A dose of 0.25 μ g vaccine stimulated minimal antibody titres after 2 weeks, but antibody was not detected thereafter. However, only 3 of 26 low-dose vaccinated mice became ill after challenge with virulent rickettsiae. In contrast, 100% morbidity and 40% mortality were observed among the non-vaccinated control mice (Table 4). Mice receiving 2.5 μ g or more of the vaccine either as single or as a divided dose, did not become ill, and no deaths were observed after challenge.

Lethality and splenomegaly in resistant and sensitive strains

Groups of resistant C57BL/6J and sensitive A/J mice were injected with mean doses ranging from $10^{-1.3}$ to $10^{10.7}$ infectious Nine Mile strain phase I *C. burnetii*. All A/J mice that received $10^{7.7}$ or more *Coxiella* died, with a mean survival time of 8 d or less (Table 5). No deaths resulted from lower doses. In contrast, C57BL/6J mice did not succumb to doses below $10^{8.7}$ micro-organisms, and doses of $10^{10.7}$ were required to kill 89% of the mice. The LD₅₀ of the A/J mice ($10^{7.1}$) was about 1000-fold lower than for the more resistant C57BL/6J mice ($10^{9.9}$).

Significant ($P < 0.05$) increases in spleen mass were observed in surviving mice of both strains 30 d after being inoculated with five or more viable rickettsiae. The degree of splenomegaly was roughly proportional to the dose received by both strains. Pathological lesions, visible as necrotic foci in the liver, were evident in 50% of the surviving C57BL/6J mice that were injected with $10^{8.7}$ or more rickettsiae. All of the A/J mice that were injected with $10^{7.7}$ or more organisms died and were not examined for necrotic lesions. Lesions were not observed in surviving A/J mice that were injected with lower doses of *C. burnetii*.

Table 5. *Survival, splenomegaly and antibody titres of A/J and C57BL/6J mice given varying doses of phase I C. burnetii*

Organisms injected*	MST, mean survival time; WCI and WCII, inactivated whole cells of phase I and phase II C. burnetii.									
	ELISA titre, log ₂ , after 30 d					ELISA titre, log ₂ , after 30 d				
	A/J		C57BL/6J			A/J		C57BL/6J		
	WCI	Antigen: WCII	LPS-I	LPS-II	Antigen: WCII	WCI	Antigen: WCII	LPS-I	LPS-II	Antigen: WCII
Spleen wt (mg) of surviving mice (mean \pm SE)†										
A/J										
C57BL/6J										
10 ^{10.7}	100 (5)	89 (8)	617 \pm 207	—	—	16.3	18.3	11.3	11.3	11.3
10 ^{9.7}	100 (7)	31 (8)	713 \pm 84	—	—	15.2	17.3	11.3	11.3	11.3
10 ^{8.7}	100 (6)	6 (9)	1199 \pm 55	—	—	17.3	17.3	11.3	11.3	11.3
10 ^{7.7}	100 (8)	0	1036 \pm 61	—	—	17.3	17.3	11.3	11.3	11.3
10 ^{6.7}	0	0	739 \pm 63	—	—	16.3	17.3	11.3	11.3	11.3
10 ^{5.7}	0	0	475 \pm 67	—	—	15.3	16.3	11.3	11.3	11.3
10 ^{4.7}	0	0	407 \pm 23	—	—	16.3	16.3	11.3	11.3	11.3
10 ^{3.7}	0	0	369 \pm 34	—	—	15.3	15.3	11.3	11.3	11.3
10 ^{2.7}	0	0	316 \pm 42	—	—	14.3	13.3	10.3	10.3	10.3
10 ^{1.7}	0	0	208 \pm 36	—	—	12.3	12.3	11.3	11.3	11.3
10 ^{0.7}	0	0	213 \pm 32	—	—	12.3	13.3	8.3	8.3	8.3
10 ^{-0.3}	0	0	96 \pm 22	—	—	12.3	12.3	8.3	8.3	8.3
10 ^{-1.3}	0	0	88 \pm 14	—	—	10.3	11.3	11.3	11.3	11.3
None	0	0	94 \pm 20	—	—	10.3	11.3	11.3	11.3	11.3

ND, Not done.

—, Not determined due to death of animals.

* Mice were given 0.5 ml i.p. containing the indicated mean dose of *C. burnetii*, phase I.

† Values for all groups that received five or more rickettsiae are significantly higher ($P < 0.05$) than for uninfected control mice.

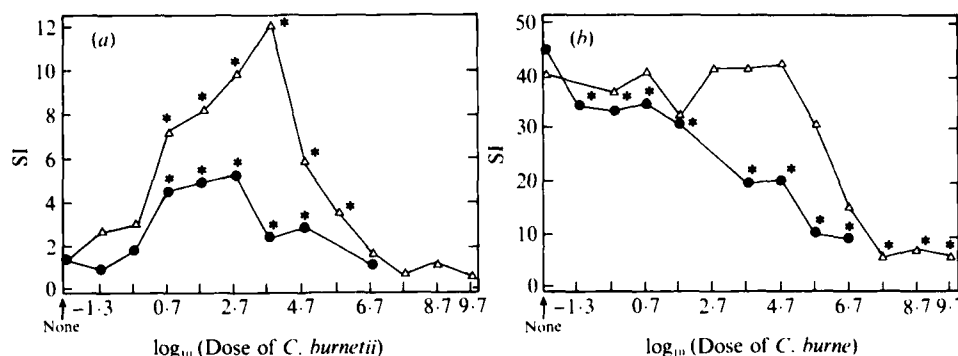


Fig. 1. *In vitro* responses of spleen cells from A/J (●) and C57BL/6J (△) mice to inactivated whole cells of phase I *C. burnetii* (a), and to Con A (b) after varying doses of phase I *C. burnetii*. Mice were given an i.p. injection containing the indicated concentrations of *C. burnetii* 30 d prior to the assay. *, Significantly different from the stimulation index (SI) of cells from uninfected mice ($P < 0.05$).

Immune response in A/J and C57BL/6J mice

Previous studies, which centred around the cellular immune responses of C57BL/10ScN mice either vaccinated or infected with phase I *C. burnetii*, indicated that pathogenic reactions were expressed as mitogenic hyporesponsiveness and antigenic negative modulation of splenic lymphocytes (Williams & Cantrell, 1981; Damrow *et al.*, 1985). Modulation of antibody production against phase I and phase II whole cells was not demonstrated. In the current study, we tested the *in vitro* proliferative responses of splenic lymphocytes and the production of specific antibodies against phase I cells, phase II cells and phase I-LPS antigens 30 d after infection of A/J and C57BL/6J mice with graded doses of phase I *C. burnetii*.

Mice of both strains that received at least one viable micro-organism developed antibody titres against phase I, phase II, and LPS antigens. Equivalent but dose-dependent titres were obtained in both strains of mice; the highest titres developed in mice that were injected with the greatest number of organisms. However, the proliferative responses of splenic lymphocytes from infected C57BL/6J mice to recall antigen were significantly higher ($P < 0.05$) than those from A/J mice for all doses up to $10^{5.7}$ micro-organisms (Fig. 1). Stimulation indices of splenic lymphocytes from C57BL/6J mice increased concomitantly with dose and were maximal in mice injected with $10^{3.7}$ organisms. Stimulation responses declined with further increases in the infecting dose; thus the proliferative response of splenic lymphocytes from C57BL/6J mice injected with $10^{6.7}$ or more rickettsiae were no higher than those of cells from uninfected mice.

Infection with low doses of *C. burnetii* also enhanced the proliferative responses of splenic lymphocytes from A/J mice to recall antigen. However, the peak response of this sensitive strain was inferior to that observed in the resistant strain, and was diminished in mice injected with more than $10^{2.7}$ rickettsiae.

Although enhanced responsiveness to recall antigen was observed after moderate infective doses of rickettsia, the Con A stimulated response of splenic lymphocytes from A/J mice injected with only one micro-organism was significantly ($P < 0.05$) suppressed below that of cells from uninfected mice. The magnitude of suppression was greater with increasing doses. By contrast, the mitogenic response of splenic lymphocytes from the resistant mouse strain was not affected by low to moderate infective doses, and was not suppressed significantly unless more than $10^{6.7}$ *C. burnetii* were injected.

DISCUSSION

In this study some differences in susceptibility and resistance among inbred strains of mice to infection by *C. burnetii* were determined. In terms of overt illness and mortality, A/J mice were the most sensitive of 47 strains surveyed. C57BL/6J mice were 1000-fold more resistant to lethal

infection than the sensitive A/J strain. A minimum dose containing $10^{7.7}$ *C. burnetii* was required to achieve 100% mortality in A/J mice; few deaths occurred in mice injected with less than $10^{6.5}$ micro-organisms. Death could not be assigned to the infection of any individual organ because *C. burnetii* were disseminated to essentially all vital organs. In eight of the strains surveyed, the infection only caused overt illness in 70–100% of the inoculated mice; these included four BALB/c strains, two 129 strains, the A/HeJ strain and the BDP/J strain. Mice in these moderately susceptible strains exhibited roughened fur and lethargy for periods of up to 17 d, and in all instances, infected mice were easily distinguished from sham-inoculated mice. As anticipated, the infection was inapparent in most of the resistant strains tested.

Comparison of known H-2 haplotypes of the strains tested according to their relative sensitivities to Q fever revealed that one (A/HeJ) of the three H-2^a strains was moderately sensitive and that two strains (A/J and A/WySnJ) were sensitive. All but one of 15 H-2^k strains were resistant. No other correlation between H-2 haplotype and sensitivity to *C. burnetii* was evident. The fact that the H-2^a strains were susceptible suggests that this locus, or the A background, or both, influence immunological interactions. Before a function to the H-2^a haplotype is assigned, genetic analyses of other factors which influence susceptibility must be considered. The A/J mouse is a low interferon producer (If-1^l), and it has deficiencies in the complement pathway (*Hc*, C5a⁻) (Cerquetti *et al.*, 1983; Gervais *et al.*, 1984; Koster *et al.*, 1985a) and in macrophage (Mac) functions (Borachi & Meltzer, 1979). Defects in macrophage-activating capacity play a role in the susceptibility of mice to other rickettsiae. However, the defect in macrophage function is not due to allelic differences at the lipopolysaccharide locus. Accumulation of deficiencies is probably responsible for the susceptibility of the A/J (C5a⁻, If-1^l, Mac⁻) and the resistance of DBA/2J (C5a⁻, If-1^h, Mac⁺) to Q fever. Although both strains of mice are deficient in secretion of C5, differences in interferon production and macrophage function may be determining factors in resistance. Gamma interferon inhibits the growth of *C. burnetii* in cultured mouse fibroblasts (Turco *et al.*, 1984). Therefore, the effects of C5, of interferon, and of macrophage function deficiencies on the susceptibility of A/J mice to *C. burnetii* require thorough study.

Examination of other genetic loci and inherent alleles for the 47 mouse strains identified a statistical correlation between the *Ighl^e* allele and sensitivity. Interestingly, the sensitive A/J strain is the prototype strain for the *Ighl^e* allele. The cellular immunological mechanisms of the *Ighl^e* allele may be significant in Q fever.

Antibody to Q fever is thought to be detrimental to the host because it facilitates uptake and placement of *C. burnetii* in the phagolysosome, thereby facilitating the infection and spread of the agent in a sensitive host (Baca *et al.*, 1984). In our experiments, both sensitive and resistant strains developed similar levels of anti-*C. burnetii* antibody after infection with only one micro-organism, yet the progression of the infection in the respective strains was apparently quite different. Thus, it appears that the quantity of anti-*C. burnetii* antibody may not be a primary factor in the clearance of *C. burnetii* from either resistant or sensitive strains.

Since cell-mediated immune responses under genetic control are important in the clearance of intracellular parasites (Rosenstreich *et al.*, 1982), we studied the *in vitro* proliferative responses of splenic lymphocytes from resistant (C57BL/6J) and sensitive (A/J) mouse strains to *C. burnetii* infections. Activated T-cells enhance the microbicidal activity of macrophages against *C. burnetii* and appear to play an important role in clearance (Kishimoto *et al.*, 1978). Our studies clearly demonstrated that low levels of *C. burnetii* infection in the sensitive A/J strain stimulated the specific *in vitro* proliferative response of splenic lymphocytes to recall antigen. In contrast, the specific response of A/J splenic lymphocytes to the T-cell mitogen Con A was suppressed, even at minimal infection levels. However, the proliferative response to recall antigens of spleen cells from resistant C57BL/6J mice were enhanced over widely varying doses of *C. burnetii* and much higher dosages (10^6 – 10^7 -fold) were tolerated without significant suppression of Con A response. Results of the lymphocyte proliferation assay to measure differences between sensitive and resistant strains suggest that the regulation of T-cell response is an important parameter in Q fever. Previous studies with mice and humans have correlated T-cell unresponsiveness with Q fever (Damrow *et al.*, 1985; Koster *et al.*, 1985a, b).

The A/J strain, with its genetic defects, is currently the best mouse model for testing the efficacy of Q fever vaccines. We have demonstrated the sensitivity of A/J mice to *C. burnetii* infection, and the ability of these mice to mount a protective immune response when vaccinated. Apparently the immunological factors which render the A/J strain sensitive were overcome by prior experience with appropriate immunogens of *C. burnetii*. The ability of sensitive mice to generate a protective immune response against lethal challenge offers a viable alternative to using the more cumbersome and expensive guinea pig model for testing candidate vaccines. Correction of immunological defects of the A/J strain as congenic constructs should provide additional clues to the importance of the *I*f-1, the *I*gh^h, and the *Mac* markers for sensitivity to Q fever.

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